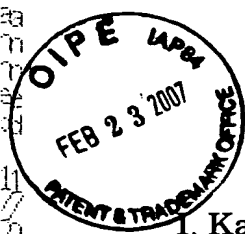


11/24/2007



VERIFICATION OF TRANSLATION

I, Kazunori Hashimoto

of 6th Fl., Kantetsu Tsukuba-Science-City Bldg. 1-1-1, Oroshi-machi,
Tsuchiura, Ibaraki, JAPAN

declare as follows:

1. That I am well acquainted with both the English and Japanese languages,
and
2. That the attached document is a true and correct translation made by me to
the best of my knowledge and belief of:-

(a) Japanese Patent Application No. Hei 10-156660

Entitled: " TRANSPORTER GENES "

Filed on May 20, 1998

September 5, 2000

(Date)

Kazunori Hashimoto

(Signature of Translator)

Kazunori Hashimoto

Patent Attorney

BEST AVAILABLE COPY

【Name of Document】 APPLICATION FOR PATENT

【Identification Number】 C2-906DP1

【Filing Date】 May 20, 1998

【Addressee】 Esq. Commissioner of the Patent Office

【International Patent Classification】 C12N 15/12

【Title of the Invention】 TRANSPORTER GENES

【Number of Claims】 12

【Inventor】

【Address】 c/o CHUGAI RESEARCH INSTITUTE FOR MOLECULAR
MEDICINE, INC. 153-2, Nagai, Niihari-mura, Niihari-gun, IBARAKI

【Name】 Jun-ichi NEZU

【Inventor】

【Address】 c/o CHUGAI RESEARCH INSTITUTE FOR MOLECULAR
MEDICINE, INC. 153-2, Nagai, Niihari-mura, Niihari-gun, IBARAKI

【Name】 Asuka OKU

【Applicant】

【Identification Number】 596102791

【Name or Appellation】 CHUGAI RESEARCH INSTITUTE FOR
MOLECULAR MEDICINE, INC.

【Representative】 Yoshiyuki Osugi

【Attorney】

【Identification Number】 100102978

【Patent Attorney】

【Name or Appellation】 Hatsushi Shimizu

【Nominated Attorney】

【Identification Number】 100108774

【Patent Attorney】

【Name or Appellation】 Kazunori Hashimoto

7860/45/11 6833805
【Priority】

【Application Number】 9-260972

【Filing Date】 September 8, 1997

【Payment】

【Registration Number】 041092

【Amount】 21000

【List of Attached Documents】

【Name of Document】 Specification 1

【Name of Document】 Drawings 1

【Name of Document】 Abstract 1

【General Power of Attorney Number】 9716403

[Document Name] Specification

[Title of the Invention] Transporter Genes

[Claims]

5 [Claim 1] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

10 [Claim 2] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 3] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

15 [Claim 4] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 4.

[Claim 5] A DNA encoding the protein according to claim 1 or 3.

20 [Claim 6] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 2.

25 [Claim 7] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO:4.

[Claim 8] A vector comprising the DNA according to any one of claims 5 to 7.

[Claim 9] A transformant expressibly carrying the DNA according to any one of claims 5 to 7.

30 [Claim 10] A method for producing the protein according to any one of claims 1 to 4, comprising the step of culturing the transformant according to claim 9.

[Claim 11] An antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:1.

35 [Claim 12] An antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[Detailed Description of the Invention]

[0001]

[Technical Field of Industrial Application]

The present invention relates to transporters, proteins
5 involved in transport of substances from the outside to the inside
of cells or vice versa.

[0002]

[Prior Art]

Recently, the involvement of various transporters localized
10 on the plasmamembrane in the uptake system for nutrients and
endogenous substances into cells and their transport mechanisms
have been clarified (Tsuji, A. and Tamai, I., Pharm. Res., 13,
963-977, 1996). These transporters recognize the structures of
substances to be transported to selectively transport specific
15 substances. Transporters that recognize the relatively wide range
of structures may possibly recognize foreign substances such as
drugs by mistake, and actively take in them into cells. It is
believed that drugs permeate through the plasmamembrane
fundamentally by simple diffusion depending on their
20 physicochemical properties such as molecular size, hydrophobicity,
and hydrogen-binding capacity. Particularly, in the case of ionic
drugs, only molecules in the non-dissociated form can permeate
through the plasmamembrane according to the pH partition hypothesis.
However, it has become evident that a number of drugs penetrate
25 through the cell membrane by a specific mechanism other than simple
diffusion, that is, an active transport mediated by transporters,
in organs that require efficient exchange of intracellular and
extracellular substances, including small intestine, uriniferous
tubule, placenta, epithelial cells of choroid plexus, hepatocytes,
30 and blood-brain barrier (Tamai, I. and Tsuji, A., Pharmacia, 31,
493-497, 1995; Saito, H. and Inui, K., Igaku no Ayumi, 179, 393-397,
1996; Tamai, I., Yakubutsu Dotai (Pharmacokinetics), 11, 642-650,
1996). For example, it is known that although oral β -lactam
antibiotics of the non-esterified type are amphoteric or negatively
35 charged in physiological pHs and sparingly soluble in fat, they
are readily absorbed through the intestine. The transport study

using the isolated membrane-vesicles system demonstrated that an H^+ -driven peptide transporter localized on the brush-border is involved in the absorption process of these drugs (Okano, T. et al., J. Biol. Chem., 261, 14130-14134, 1986). Although the specificity of a peptide transport system in terms of the peptide size is so strict as to recognize di- or tri-peptides but not tetrapeptides or larger peptides, it has a rather broad substrate specificity to recognize peptides comprising non-natural amino acids. The peptide transporter mediates transport of β -lactam antibiotics mistakenly due to its broad substrate specificity. This property has been unexpectedly utilized in the clinical field (Tsuji, A., American Chemical Society (eds. Taylor, M. D., Amidon, G. L.), Washington, D. C., 101-134, 1995). Furthermore, a possibility that a transporter is also involved in permeation of substances with a high hydrophobicity such as fatty acids through the plasmamembrane has been reported (Schaffer, J. and Lodish, H., Cell, 79, 427-436, 1994).

[0003]

Since various transporters are supposed to be distributed in organs and cells based on the physiological roles of the organs and cells, their distribution and functions may be specific to organs. Therefore, transporters are expected to be used to impart an organ specificity to pharmacokinetics. In other words, an organ-specific drug delivery system (DDS) can be constructed utilizing transporters. If drug absorption solely relied on simple diffusion is improved by elevating its hydrophobicity, an effect of the drug obtained in the initial transport in the liver can be enhanced and the drug can non-specifically translocate into any organ. In addition, it would also be possible to increase the drug absorption independently of its fat-solubility by designing the drug utilizing the substrate specificity of transporters (Hayashi, K. et al., Drug Delivery System, 11, 205-213, 1996). For this purpose, it is necessary to identify various transporters at the molecular level and analyze their properties in detail. However, their molecular level identification are greatly behind studies on their membrane physiology because they

are difficult to handle biochemically and require complicated processes in their functional assays.

[0004]

Recently, cDNAs of several transporters have been cloned by the expression cloning method using *Xenopus* oocytes, a foreign gene expression system, and structural homology among them has been revealed (Fei, Y.-J. et al., *Nature*, 368, 563-566, 1994). For example, Koepsell et al. cloned an organic cation transporter, OCT1, which is assumed to be localized on a basement membrane, using the expression cloning method in 1994 (Grundemann, D. et al., *Nature*, 372, 549-552, 1994). Subsequently, OCT2 was identified by homology cloning based on the sequence of OCT1 (Okuda, M. et al., *Biochem. Biophys. Res. Commun.*, 224, 500-507, 1996). OCT1 and OCT2 show homology as high as 67% to each other, but they differ in their distribution at uriniferous tubules, and OCT2 is believed to be mostly localized at luminal side (Grundemann, D. et al., *J. Biol. Chem.*, 272, 10408-10413, 1997). Both of them are intensely expressed in the kidney, but differ in the organ distribution; OCT1 is also expressed in the liver, colon, and small intestine, while OCT2 expression is specific to the kidney.

[0005]

Only a few reports on identification of transporters at the molecular level, including the reports, are available, and there would be many unknown transporters that may be clinically useful.

[0006]

[Problems to Be Solved by the Invention]

An object of this invention is to provide a novel transporter gene and its protein.

[0007]

[Means to Solve the Problems]

The present inventors have screened a fetal gene library constructed using the subtractive method by random sequencing based on a working hypothesis that fetal genes include those which are involved in various disorders including cancer and are specifically or intensely expressed in fetal tissues. The inventors discovered an unknown gene showing a significant homology with those for organic

cation transporters, OCT1 and OCT2, and attempted to isolate this gene, which was assumed to encode a novel transporter. Thus, the inventors succeeded in isolating the desired gene by screening a cDNA library derived from human fetus. Furthermore, the inventors studied the transporter activity of a protein encoded by the isolated human gene and found that the protein, in fact, functioned as a transporter for various organic cations.

[0008]

This invention relates to a novel transporter, and more specifically to:

(1) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added;

(2) a protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2;

(3) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added;

(4) a protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 4;

(5) a DNA encoding the protein according to (1) or (3);

(6) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2;

(7) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO:4;

(8) a vector comprising the DNA according to any one of (5) to (7);

(9) a transformant expressibly carrying the DNA according to any one of (5) to (7);

(10) a method for producing the protein according to any

one of (1) to (4), comprising the step of culturing the transformant according to (9);

(11) an antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:1; and

(12) an antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[0009]

[Mode for Carrying Out the Invention]

This invention relates to a novel transporter protein.

Nucleotide sequences of cDNAs of novel human transporters isolated by the present inventors are shown in SEQ ID NO: 2 and SEQ ID NO: 4, respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NO: 1 and SEQ ID NO: 3, respectively. Amino acid sequences of these two proteins included in the transporter proteins of this invention showed such a high overall homology as about 76%, and both of them retained the following consensus sequence which is conserved in various types of transporters including the glucose transporter: [Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala] (Maiden, M. C. et al., Nature, 325, 641-643, 1987). In fact, these proteins have an activity to transport various organic cations.

[0010]

Transporter proteins of this invention also include those having the additional activity to transport substances other than organic cations as far as they retain the organic cation transport activity. Organic cations include, for example, TEA, carnitine, quinidine, and pyrilamine, but are not limited to them. They also include carcinostatic agents such as actinomycin D, etoposide, vinblastine, daunomycin, etc. Transporter proteins of this invention include those having the activity to transport organic cations not only from the outside to the inside of cells but also from the inside to the outside of cells.

[0011]

Transporter proteins of this invention can be prepared as recombinant proteins using recombination techniques or natural proteins. Recombinant proteins can be prepared, for example, as described below, by culturing cells transformed with DNA encoding proteins of this invention. Natural proteins can be isolated from the kidney and cancer cell strains such as Hela S3, which highly express the proteins of this invention, by the method well known to those skilled in the art, for example, affinity chromatography using an antibody of this invention described below. The antibody may be either polyclonal or monoclonal. A polyclonal antibody can be prepared by purifying serum obtained from, for example, a small animal such as a rabbit immunized with proteins of this invention by known methods, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. A monoclonal antibody can be prepared by immunizing a small animal such as a mouse with the protein of this invention, excising the spleen from the mouse, grinding the tissue into cells, fusing them with mouse myeloma cells using a fusing agent such as polyethyleneglycol, and selecting a clone that produces an antibody to proteins of this invention out of fused cells (hybridomas) thus produced. Then, hybridomas thus selected are transplanted into the abdominal cavity of a mouse, and the ascites is collected from the mouse. A monoclonal antibody thus obtained can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. When the antibody thus obtained is administered to human subjects, a humanized antibody or a human antibody is advantageously used to reduce the immunogenicity. An antibody can be humanized by, for example, the CDR grafting method comprising cloning an antibody gene from monoclonal antibody-producing cells and grafting the epitope portion thereof into an existing human antibody. A human antibody can be prepared by the usual method for preparing a monoclonal antibody except for immunizing a mouse whose immune system is replaced with the

human's.

[0012]

It is also possible for those skilled in the art to prepare proteins having functions equivalent to the transporter proteins set forth in SEQ ID NOS: 1 or 3 by appropriately modifying amino acid residues of the proteins by, for example, substitution, using well known methods. Mutation of amino acids of the proteins can occur also spontaneously. Such mutant proteins which are obtained by altering the amino acid sequence of the proteins set forth in SEQ ID NOS: 1 or 3 by substitution, deletion, or addition of amino acid residues, and are functionally equivalent to those of the proteins set forth in SEQ ID NOS: 1 or 3 are also included in the proteins of this invention. Herein, "functionally equivalent" means that proteins have an activity to transport organic cations. Methods well known to those skilled in the art for altering amino acids include, for example, the site-specific mutagenesis system by PCR (GIBCO-BRL, Gaithersburg, Maryland), site-specific mutagenesis by oligonucleotide (Kramer, W. and Fritz, H. J. (1987) Methods in Enzymol., 154: 350-367), Kunkel's method (Methods Enzymol., 85, 2763-2766 (1988)), etc. The number of amino acids that can be substituted is usually 10 amino acid residues or less, preferably 6 or less, and more preferably 3 or less. The site of substitution, deletion, or addition of amino acid residues is not particularly limited as far as the activity of proteins of this invention is retained. It is possible to detect the transporter activity of proteins, for example, by the method described below in Example 6.

[0013]

It is routine for those skilled in the art to obtain proteins functionally equivalent to the proteins of this invention by isolating and using DNAs highly homologous to the DNA sequences set forth in SEQ ID NOS: 2 or 4 or portions thereof using hybridization techniques (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989), etc. Therefore, it is possible for those skilled in the art to prepare proteins which are coded by the DNA hybridizing to DNA of SEQ ID NOS: 2 or

4 and are functionally equivalent to the transporter proteins of SEQ ID NOS: 1 or 3. These proteins are also included in proteins of this invention. Here, "functionally equivalent" means that proteins have an activity to transport organic cations, as mentioned above. DNAs that hybridize to the DNAs encoding the proteins of this invention can be isolated from other organisms, for example, mice, rats, rabbits, cattle, etc. Especially, tissues such as the kidney are suitable as sources of such DNAs. These DNAs thus isolated which encode the proteins having equivalent function to the proteins set forth in SEQ ID NOS: 1 or 3 usually have a high homology with the DNAs set forth in SEQ ID NOS: 2 or 4. "High homology" means at least 70% or more, preferably at least 80% or more, and more preferably at least 90% or more of sequence identity with DNAs set forth in SEQ ID NOS: 2 or 4.

[0014]

One example of hybridization conditions for isolating such DNAs is as follows. That is, after the pre-hybridization at 55°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 37°C to 55°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS once at 37°C for 20 min. More preferable conditions are as follows. After the pre-hybridization at 60°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 60°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS twice at 50°C for 20 min. Still more preferable conditions are as follows. After pre-hybridization at 68°C for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 68°C for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 0.1 x SSC and 0.1%

SDS twice at 50°C for 20 min.

[0015]

The present invention also relates to DNAs encoding the above-described transporter proteins of this invention. DNAs of this invention may be cDNA, genomic DNAs, and synthetic DNAs. The DNAs of the present invention can be used for producing proteins of this invention as recombinant proteins, as well as for isolating proteins which are functionally equivalent to proteins of SEQ ID NOS:1 or 3 . That is, it is possible to prepare proteins of this invention as recombinant proteins by inserting DNAs encoding proteins of this invention (e.g. DNAs set forth in SEQ ID NOS: 2 or 4) into an appropriate expression vector, culturing transformants obtained by transfecting suitable cells with the vector, and purifying the proteins thus expressed. Cells to be used for producing recombinant proteins include, for example, mammalian cells such as COS cells, CHO cells, NIH3T3 cells, etc., insect cells such as Sf9 cells, yeast cells, *E. coli*, and so on. Vectors used for the intracellular expression of recombinant proteins vary depending on host cells, including, for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids Res., 1990, 18(7), p5322), etc. for mammalian cells, "BAC-to-BAC baculovirus expression system" (GIBCO BRL), etc. for insect cells, "Pichia Expression Kit" (Invitrogen), etc. for yeast cells, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), etc. for *E. coli*. Host cells can be transformed with vectors, for example, by the calcium phosphate method, the DEAE-dextran method, the method using cationic liposome DOTAP (Boehringer Mannheim), the electroporation method, the calcium chloride method, etc. Recombinant proteins can be purified from recombinants thus obtained using standard methods, for example, as described in "The Qiaexpressionist Handbook, Qiagen, Hilden, Germany."

[0016]

The DNAs of this invention can be used in gene therapy for disorders caused by abnormalities in the activity and expression of the proteins of this invention. In this case, the DNAs of this invention are inserted into an adenovirus vector (e.g. pAdexLcw),

a retrovirus vector (e.g. pZIPneo), etc., and administered into the living body by either *ex vivo* method or *in vivo* method. Gene therapy can also be performed by administering a synthetic antisense DNA to the living body directly or after inserted into the
5 above-described vectors.

[0017]

The transporter proteins of this invention can be used to control internal absorption and dynamics of drugs. Based on the results of detailed analysis of the substrate specificity of
10 transporter proteins of this invention, drugs can be designed so as to be transported by these transporters and absorbability of the drugs mediated by these transporter proteins can be improved. Conventional modifications to enhance hydrophobicity are no longer necessary for drugs so designed, which enables speedily and
15 efficiently developing water-soluble drugs that are easy to handle. The drugs thus developed are thought to be absorbed principally depending on the internal distribution pattern of transporter proteins of this invention, and an organ-specific delivery of the drugs thus becomes possible. Especially, if the transporter
20 proteins of this invention are distributed in the target organ of a drug, an ideal drug delivery system (DDS) can be developed. If a drug is to be absorbed mediated by not the transporter proteins of this invention but other transporters, the drug can be designed so as to be specific to other transporter proteins by designing
25 it considering the substrate specificity of the transporter proteins of this invention. Since the transporter proteins of this invention are present in the kidney, it is possible to reduce the nephrotoxicity produced by a drug by designing the drug so that it can be readily excreted by the transporter proteins of
30 this invention.

[0018]

Another possible application of this invention is to develop a drug targeting the transporter proteins of this invention. The transporters play important roles in the absorption mechanism of
35 nutrients and drugs, or the excretion mechanism of drugs and internal metabolites. Thus, damage or abnormal elevation of the

transporter's functions may cause some disorders. It is considered to be efficacious against such disorders to administer a drug that inhibits or enhances functions of the transporter proteins of this invention, or regulates the expression level of the transporter gene of this invention and the amount of the transporter proteins. The gene therapy above-mentioned is also efficacious.

[0019]

Furthermore, since "OCTN2" included in the transporter proteins of this invention efficiently transports carnitine, chemotherapy with compounds to control the activity of "OCTN2" or gene therapy using the "OCTN2" gene is considered to be efficacious against various pathological conditions such as fatty liver, etc. caused by hypocarnitinemia.

[0020]

The transporter proteins of this invention are expressed in a variety of cancer cell strains, which suggests that the proteins may transport drugs into tumor cells. If this is the case, it is possible to develop carcinostatics that will be readily absorbed mediated by the transporter proteins of this invention. On the contrary, mechanisms to transport and excrete substances by the transporter proteins of this invention may function to excrete carcinostatics in tumor cells so that the cells acquire resistance to drugs. If the transporter proteins of this invention are involved in a mechanism of tumor cells to acquire drug resistance, a carcinostatic effect can be enhanced by a combined use of inhibitors of the transporter proteins of this invention with carcinostatics.

[0021]

The present invention is described below in more detail with reference to examples, but is not construed being limited thereto.

[0022]

[Examples]

Example 1 Construction of a subtraction library

A subtraction library was constructed using the PCR-Select™ cDNA Subtraction Kit (CLONTECH) principally according to the method of Luda Diatchenko (Diatchenko, L. et al., Proc. Natl. Acad. Sci.

USA, 93, 6025-6030, 1996).

[0023]

First, double-stranded cDNAs were synthesized from poly(A)⁺ RNAs derived from human fetal liver and adult liver by the standard method using MMLV reverse transcriptase. These cDNAs were blunt-ended with T4 DNA polymerase and cleaved with RsaI. A part of the cDNAs derived from fetal liver (tester) was divided in two portions, and they were separately ligated to two different adapters, adapter 1 and adapter 2, respectively (Table 1). A 120-fold excess of cDNA derived from adult liver (driver) was added to each of the above-described tester samples. The mixture was heat-denatured and subjected to the primary hybridization at 68°C for 8 h. After these two reaction mixtures from the primary hybridization were mixed together without heat-denaturation, an excessive amount of the heat-denatured driver was further added thereto, and the mixture was subjected to the secondary hybridization at 68°C for about 16 h. The resulting reaction solution was diluted with a dilution buffer and incubated at 75°C for 7 min. After the shorter strands of adapters were removed, the reaction solution was used as a template for PCR. PCR using primers 1 (5'-CTAATACGACTCACTATAGGGC-3', SEQ ID NO: 5) and 2 (5'-TGTAGCGTGAAGACGACAGAA-3', SEQ ID NO: 6) corresponding to the adapters selectively amplified only cDNAs having different adapters at their both ends (subtracted cDNAs) (suppression PCR). PCR was carried out using a portion of the resulting cDNA as a template, and nested PCR primers 1 (5'-TCGAGCGGCCGCCCCGGGCAGGT-3', SEQ ID NO: 7) and 2 (5'-AGGGCGTGCTGCGGAGGGCGGT-3', SEQ ID NO: 8), which are further inwardly located from the PCR primers 1 and 2, to obtain products with further elevated selectivity. PCR products thus obtained were purified using the QIAquick PCR Purification kit (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to construct a subtraction library.

[0024]

[Table 1]

Adapter 1	5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'
--------------	---

Adapter 2	5'-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTGCGGAGGGCGGT-3' 3'-GCCTCCCGCCA-5'
--------------	--

Example 2 cDNA cloning

To analyze fetal genes, the subtraction library derived from the fetal liver was screened by random sequencing. Homology search (Blastx) of Expressed Sequence Tags (ESTs) thus obtained found a clone, OCTN1 (fls 631) (292 bp) encoding amino acid sequence having significant homology with the known organic cation transporters, OCT1 (Grundemann, D. et al., Nature, 372, 549-552, 1994) and OCT2 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996). Since the sequence of this clone was novel and assumed to be a fragment derived from a new transporter gene, cDNA comprising the whole open reading frame (ORF) of this gene was cloned.

[0025]

The human fetal liver 5'-stretch cDNA library (CLONTECH) was screened using the original OCTN1 clone obtained from the subtraction library derived from fetal liver as a probe. An insert of the original OCTN1 clone was amplified by PCR using M13 P4-22 and M13 P5-22, and labeled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was carried out at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. Screening about 5 x 10⁵ phage clones finally isolated seven positive clones. cDNA inserts of these clones were amplified by PCR using vector primers designed based on a sequence of the λ gt10 vector (GT10 S1 5'-CTTTTGAGCAAGTTCAGCCT-3', SEQ ID NO: 9, and GT10 A1 5'-AGAGGTGGCTTATGAGTATTTCTT-3', SEQ ID NO: 10), or primers designed based on the decoded cDNA sequences. The PCR products thus obtained were directly sequenced to determine the nucleotide sequences. Some regions that were difficult to be amplified were subjected to PCR using 7-deaza dGTP as a substrate base (McConlogue, L. et al., Nucleic Acids Res., 16, 9869, 1988).

[0026]

After screening about 5×10^5 phage clones, finally seven positive clones were isolated. Sequencing of cDNA inserts of these clones revealed that the OCTN1 gene contains an ORF encoding a protein consisting of 551 amino acid residues (putative molecular weight of about 62,000). Data base search using this whole amino acid sequence confirmed that it has a significant overall homology (about 34%) with OCT1 and OCT2. Hydrophobicity profile of this sequence obtained by Kyte & Doolittle's calculating formula (Kyte, J. and Doolittle, R. F., J. Mol. Biol., 157, 105-132, 1982) very closely resembled those of OCT1 and OCT2, indicating that the sequence has eleven to twelve putative transmembrane hydrophobic regions (Fig. 1). This sequence contained one consensus sequence of sugar transporter, ([Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala]), (160 to 175). This consensus sequence is present in the glucose transporters GLUT1 to GLUT7 in mammalian cells, and also present in various types of transporters other than glucose transporters (Maiden, M. C. et al., Nature, 325, 641-643, 1987). Furthermore, putative N-linked glycosylation sequences (N-X-[ST]) were found in the amino acid sequence of OCTN1 at four sites (57 to 59, 64 to 66, 91 to 93, and 304 to 306), and also five putative protein kinase C phosphorylation sites ([ST]-X-[RK]) (164 to 166, 225 to 227, 280 to 282, 286 to 288, and 530 to 532). In addition, the consensus sequence ([Ala, Gly]-Xaa(4)-Gly-Lys-[Ser, Thr]) of the ATP/GTP binding site is also found. This consensus sequence of the ATP/GTP binding site is also present in the ATP binding protein or GTP binding protein, such as kinases and ras family proteins, and that ATP or GTP binds to this site (Walker, J. E. et al., EMBO J., 1, 945-951, 1982). This sequence is present in the so-called ATP Binding Cassette (ABC) type transporter, and involved in the substance transport using the energy generated by hydrolysis of ATP (Higgins, C. F. et al., J. Bioenerg. Biomembr., 22, 571-592, 1990; Urbatsch, I. L. et al., J. Biol. Chem., 270, 26956-26961,

1995). Presence of this consensus sequence indicates that OCTN1 protein may be an ATP or GTP-dependent transporter.

[0027]

Nucleotide sequencing was performed by the cycle-sequencing method with a plasmid DNA prepared by the alkaline-SDS method or a PCR product obtained by colony PCR, etc. as a template using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, followed by decoding with the ABI 377 DNA Sequencer (Perkin Elmer). Colony PCR was carried out by directly suspending a colony of a recombinant in a PCR reaction solution containing vector primers M13 P4-22 (5'-CCAGGGTTTCCAGTCACGAC-3', SEQ ID NO: 11) and M13 P5-22 (5'-TCACACAGGAAACAGCTATGAC-3', SEQ ID NO: 12). After the completion of PCR, a DNA insert thus amplified was separated from unreacted primers and nucleotides by gel filtration, etc. to serve as a template for sequencing.

[0028]

Example 3 Northern analysis

Distribution of OCTN1 in tissues was investigated by Northern analysis (Fig. 2). A 3'-end fragment of OCTN1 (the latter half from around the base 1,100) was labelled with [α -³²P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was performed using the Multiple Tissue Northern (MTN) Blot - Human, Human III, Human IV, Human Fetal II, and Human Cell lines (CLONTECH) at 68°C in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50°C in 0.1 x SSC and 0.1% SDS. As a result, RNA of about 2.5 kb was strongly expressed in the fetal liver and adult-derived tissues such as the kidney, bone marrow, and trachea. Besides those tissues, the RNA band was also weakly detected in the fetal kidney and lung, and adult tissues including skeletal muscle, lung, placenta, prostate, spleen, and spinal cord. The RNA expression was also detected in tumor cell lines such as HeLa S3, K562, SW480, and A549, and especially, its very intense expression was observed in HeLa S3.

[0029]

Example 4 Cloning of OCTN2 cDNA

Data base search using the entire nucleotide sequence of "OCTN1" detected very similar sequences thereto in several parts of the nucleotide sequence of P1 phage clones (P1 H24 clones, GenBank accession No. L43407, L43408, L46907, L81773, and L43409) derived from q regions of human chromosome 5. The parts having similarity with the nucleotide sequence of OCTN1 are separated by the sequences having no similarity to the OCTN1 sequence. The sequence obtained by connecting these similar parts with each other with reference to the sequence of OCTN1 has a high homology over a wide range with OCTN1, indicating the presence of OCTN1 homologues. The genomic sequence registered in data base was an incomplete one without covering the entire coding region, and, from only this sequence, it was impossible to know the complete structure of a protein partially encoded by the sequence. Therefore, cDNA cloning of this OCTN1 homologous gene (OCTN2) was performed to determine the coded protein structure. First, 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631RA1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) were prepared based on sequences of these P1 phage clones. PCR was performed using a set of these primers and cDNA synthesized from poly(A)⁺ RNA derived from the human adult kidney (CLONTECH) as a template, under the following conditions: 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min,; and 1 cycle of 72°C for 10 min, resulting in amplification of about 900 bp fragment. This fragment was subcloned into the pT7Blue-T vector (Novagen) by the TA cloning method to determine its nucleotide sequence, which clearly showed a very high overall homology with OCTN1. Therefore, this gene was designated as OCTN2, and longer cDNAs were cloned.

[0030]

The cDNA library derived from the human kidney was screened using the cDNA insert of this clone as a probe in the same manner as for OCTN1 cDNA cloning, and cDNA containing the entire coding region of OCTN2 was cloned by a procedure for isolating longer

clone and the Rapid Amplification of cDNA Ends (RACE) method (Chenchik, A., Moqadam, F., and Siebert, P. (1995), CLONTECHniques X, 5-8), etc. to determine its structure (SEQ ID NO: 4). Specifically, the RACE method was carried out as follows. The 631R S6 primer (5'-AGCATCCTGTCTCCCTACTTCGTT-3', SEQ ID NO: 15) was prepared. PCR was performed using this primer and the Marathon-Ready™ cDNA derived from the human adult kidney (CLONTECH) as a template under the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 3 min,; and 72°C for 10 min, resulting in amplification of about 1.7 kbp cDNA fragment of the 3'-end. This fragment was subcloned into the pT7Blue-Tvector by the TA cloning method to determine its structure.

[0031]

It became evident that OCTN2 contains an open reading frame (ORF) encoding a protein consisting of 557 amino acid residues. Fig. 3 compares amino acid sequences of OCTN1 and OCTN2. Both showed overall amino acid homology as high as about 76%. In addition, one consensus sequence (160 to 176) of sugar transporter was present in the amino acid sequence of OCTN2 like OCTN1. These facts indicated that OCTN2 can be a novel transporter that is structurally related to OCTN1. Furthermore, a consensus sequence (218 to 225) of the ATP/GTP binding site was also present in the amino acid sequence of OCTN2 like in OCTN1.

[0032]

Example 5 Northern analysis

Northern analysis was performed using about 900 bp OCTN2 cDNA as a probe which was obtained by PCR with a set of 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) in the same manner as for OCTN1. The results are shown in Fig. 4. Although the expression pattern of OCTN2 partly overlapped with that of OCTN1, OCTN2 differs from OCTN1 in that the former was very intensely expressed in the kidney among fetal tissues, while the latter was strongly expressed also in cancer cell strains such as K-562, HeLa S3, SW480, etc. as well as the kidney, indicating that OCTN1 and OCTN2 may be involved in transport of substances such as

carcinostatics in these cancer cells.

[0033]

Example 6 Forced expression of OCTN1 in human fetal kidney cells (HEK293) and its activity determination

5 Phage DNAs were extracted from positive phage clones obtained by screening the clones by the plaque hybridization method using the QIAGEN Lambda Kit (QIAGEN). After the DNA insert was subcloned into the pUC18 vector, cDNA containing the entire ORF which was cleaved out with SmaI and EcoRI was integrated between the EcoRI
10 site and the blunted HindIII site of an expression vector for mammalian cells, pCDNA3 (Invitrogen), to obtain an expression plasmid DNA, pCDNA3/OCTN1. Plasmid DNA was prepared by alkaline-SDS method using the QIAGEN PLASMID MAXI Kit (QIAGEN).

[0034]

15 The human fetal kidney-derived cell strain, HEK 293 cells were transfected with the plasmid pCDNA3/OCTN1 and pCDNA3 vector containing no insert as a control by the calcium phosphate method. First, the plasmid DNA (10 μ g), a Hepes buffer solution (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 6 mM Dextrose, and 21 mM Hepes pH
20 7.1) (1 ml), and 2M CaCl_2 (62.5 μ l) were combined and allowed to stand at room temperature for 30 min or more to form calcium phosphate coprecipitates. After cells were plated on 10-cm plates at 1.5×10^6 cells per plate and cultured for 24 h, the calcium phosphate coprecipitates were added thereto, and the cells were further
25 cultured for 24 h. Then, plates were washed with phosphate buffered saline (PBS), and the cells were further cultured for 24 h after the addition of fresh culture medium.

[0035]

30 Transport experiment was performed using cells transfected with the plasmid DNA or untreated cells according to the following procedures. Cells were detached from plates using a rubber policeman, suspended in a transport buffer (containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM (+)-glucose, 1.2 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , and 25 mM Hepes pH 7.4), and pre-incubated for 20 min.
35 An appropriate amount of each labeled substrate ($[^{14}\text{C}]\text{TEA}$ (tetraethylammonium) (NEN), $[^3\text{H}]\text{carnitine}$ (L-carnitine

hydrochloride) (Amersham), [³H]PCG (benzylpenicillin) (Amersham), [³H]quinidine (ARC), or [³H]pyrilamine (mepyramine) (Amersham)) was then added to the cell suspension, and the resulting mixture was incubated at 37°C for a predetermined period of time. Incubated cells were overlaid on a silicon layer prepared by layering a mixture of silicon oil and liquid paraffin (specific gravity = 1.022) on a 3 M KCl layer, and separated by centrifugation. Radioactivity of cells was measured to determine the into-the-cell transport activity. In this case, 1 x 10⁶ cells were used as one point of cells. HEK 293 cells were cultured in Dulbecco's MEM containing 10% fetal calf serum (FCS) in an atmosphere of 5% carbon dioxide at 37°C.

[0036]

First, the transporter capacity was measured in the cells transfected with pCDNA3/OCTN1 and untreated cells using TEA as a substrate (Fig. 5). A reaction time-dependent TEA uptake into the OCTN1-transfected cells was clearly observed. This uptake was not observed in untreated cells. Next, effects of the addition of unlabeled TEA on the labeled substrate uptake in this system (cold inhibition) were examined (Fig. 6). A decrease in the apparent uptake of the labeled substrate was clearly seen depending on the concentration of cold TEA added. In this experiment, almost no uptake of the substrate into cells was observed in cells transfected with the pCDNA3 vector containing no insert (Mock) used as a control like in untreated cells used, clearly indicating that this uptake phenomenon is due to the transfection of the cells with OCTN1. Next, to obtain the K_m (Michaelis constant) value of OCTN1 to TEA, the uptake of ¹⁴C-TEA with various concentrations was measured (Fig. 7). From Lineweaver-Burk reciprocal plot of the net uptake obtained by subtracting the amount of the uptake in Mock cells from that in the human OCTN1-transfected cells, the K_m value of 0.44 ± 0.04 mM was obtained with the maximal velocity, V_{max} of 6.68 ± 0.34 (nmol/3 min/mg). Next, the transport capacity of OCTN1 for other substrate than TEA was examined (Fig. 8). When the transport capacity was measured using labeled organic cations such as labeled carnitine, quinidine, and pyrilamine, a significant

increase in the uptake of these compounds was clearly observed in OCTN1-transfected cells as compared with Mock cells, clearly indicating that these organic cations can serve as substrates for OCTN1. However, no significant increase in the uptake of an organic anion, PCG (benzylpenicillin), was observed.

[0037]

Example 7 Activity measurement of OCTN1 using *Xenopus* oocytes
cRNA was synthesized *in vitro* using T7 RNA polymerase with pcDNA3/OCTN1 as a template. This cRNA was diluted to the concentration of 0.3 ng/nl, and its 50-nl (15 ng) aliquot was injected into a single oocyte. As a control, 50 nl of distilled water was injected. These oocytes were cultured for 3 days, and then used for the transport experiment. After being preincubated in an uptake buffer (0.05% Tween 80, 100 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM Hepes pH 7.4) at 25°C for 20 min, the oocytes were transferred to the uptake buffer containing 0.5 ml of labeled substrate to initiate the uptake. After the incubation at 25°C for 1 h, the oocytes were washed in the ice-cold uptake buffer three times to terminate the reaction. The oocytes were solubilized in 5% SDS and mixed with Cleasol I (a cocktail for liquid scintillation counter) (3 ml) to determine the radioactivity. The radioactivity of the uptake buffer which contained the labeled compound at the time of incubation (external solution) (10 μ l) was also similarly measured. The ratio of the radioactivity (dpm value) in the oocytes to that (dpm value) in the external solution was used as the uptake activity.

[0038]

OCTN1 also expresses the transport capacity for organic cations such as quinidine, mepyramine and carnitine, as well as TEA in this transport experiment system using *Xenopus* oocytes (Fig. 9).

[0039]

Next, the transport capacity of OCTN1 for carcinostatics, etc. was examined. The results revealed that human OCTN1 has the activity to transport actinomycin D, etoposide, vinblastine, and daunomycin (Fig. 10). These results strongly indicate that OCTN1

would be involved in the into-the-cell translocation mechanism (mechanism for absorption by cells) for these drugs, which have been clinically used as carcinostatics. By designing and screening drugs utilizing the substrate specificity of OCTN1 so as to be readily recognized by this transporter, it would be possible to efficiently develop useful drugs that can be readily absorbed by the cells.

[0040]

Example 8 Forced expression of OCTN2 in HEK cells and its activity measurement

The expression plasmid DNA for OCTN2 in mammalian cells was prepared as follows.

A single-stranded cDNA was synthesized from poly(A)⁺ RNA derived from the human fetal kidney (CLONTECH) using the SuperScript[™] II reverse transcriptase (GIBCO BRL). PCR was performed using the thus-obtained cDNA as a template under the following conditions to amplify 5'- and 3'-end fragments of human OCTN2.

[0041]

For the amplification of 5'-end fragment (about 800 bp) of OCTN2, OCTN2 3 primer (5'-GATGGATCCCGGACGGTCTTGGGTCGCCTGCTG-3', SEQ ID NO: 16) and OCTN2 4 primer (5'-GATGGATCCAAATGCTGCCACATAGTTGGAGAT-3', SEQ ID NO: 17) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (150 μ M 7-deaza dGTP, 50 μ M dGTP, 200 μ M dATP, 200 μ M dTTP, and 200 μ M dCTP) according to the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 2 min,; and 72°C for 10 min. For the amplification of 3'-end fragment (about 1.2 kbp) of OCTN2, OCTN2 7 primer (5'-GATGGATCCATGGGCATGCAGACAGGCTTCAGC-3', SEQ ID NO: 18) and OCTN2 8 primer (5'-GATGGATCCTTCCTCTTCAGTTTCTCCCTTACT-3', SEQ ID NO: 19) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (200 μ M dGTP, 200 μ M dATP, 200 μ M dTTP, and 200 μ M dCTP) according to the following conditions: 94°C for 2 min; 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 2 min,; and 72°C for 10 min.

[0042]

These fragments were respectively electrophoresed on agarose gel, excised from the gel, purified, and subcloned into the pT7Blue-T vector. Clones having no PCR error were selected by sequencing, and clones from both fragments were ligated at the PstI site in the overlapping region. Each ligated fragment was eventually incorporated into the BamHI site of the pcDNA3 vector, and used as the expression plasmid DNA pcDNA3/OCTN2.

[0043]

HEK cells were transfected with pcDNA3/OCTN2, the pcDNA3 vector containing no insert (Mock), or pcDNA3/OCTN1 by the method described in Example 6 to perform transport experiments. It was proved that OCTN2 has a high capacity to efficiently transport carnitine (Fig. 11). On the other hand, OCTN2 hardly transported TEA, which were efficiently transported by OCTN1, revealing that they clearly differ in their substrate specificities.

[0044]

Next, Na^+ dependence of OCTN2-mediated carnitine transport was examined using a transport buffer in which Na^+ was replaced with K^+ (Fig. 12). The result showed that carnitine transport mediated by OCTN2 completely depended on the presence of Na^+ , indicating that OCTN2 is a symport type transporter that transports substrates and Na^+ in the same direction.

[0045]

[Effects of the Invention]

This invention provides a novel organic cation transporter gene and proteins. Transporter of this invention are useful for developing newly designed drugs that can be transported mediated by these proteins, and pharmaceuticals for disorders caused by functional abnormalities of the proteins.

Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val
 140 145 150
 Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg
 155 160 165
 5 Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe
 170 175 180 185
 Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val
 190 195 200
 Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu
 10 205 210 215
 Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu
 220 225 230
 Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe
 235 240 245
 15 Ala Tyr Phe Ile Arg Asp Trp Arg Met Leu Leu Leu Ala Leu Thr Val
 250 255 260 265
 Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro
 270 275 280
 Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile
 20 285 290 295
 Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe
 300 305 310
 Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile
 315 320 325
 25 Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser
 330 335 340 345
 Leu Leu Leu Trp Met Leu Thr Ser Val Gly Tyr Phe Ala Leu Ser Leu
 350 355 360
 Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser
 30 365 370 375
 Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg
 380 385 390
 Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly
 395 400 405
 35 Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu
 410 415 420 425

Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
430 435 440
Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
445 450 455
5 Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile
460 465 470
Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
475 480 485
Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
10 490 495 500 505
Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
510 515 520
Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
525 530 535
15 Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
540 545 550

SEQ ID NO: 2

SEQUENCE LENGTH: 2135

20 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

FEATURE

25 NAME/KEY: CDS

LOCATION: 147.. 1799

IDENTIFICATION METHOD: E

SEQUENCE

CCCCGGCTTC GCGCCCAAT TTCTAACAGC CTGCCTGTCC CCCGGAACG TTCTAACATC 60
30 CTTGGGGAGC GCCCCAGCTA CAAGACACTG TCCTGAGAAC GCTGTCATCA CCCGTAGTTG 120
CAAGTTTCGG AGCGGCAGTG GGAAGC ATG CGG GAC TAC GAC GAG GTG ATC GCC 173
Met Arg Asp Tyr Asp Glu Val Ile Ala
1 5
TTC CTG GGC GAG TGG GGG CCC TTC CAG CGC CTC ATC TTC TTC CTG CTC 221
35 Phe Leu Gly Glu Trp Gly Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu
10 15 20 25

	AGC GCC AGC ATC ATC CCC AAT GGC TTC AAT GGT ATG TCA GTC GTG TTC	269
	Ser Ala Ser Ile Ile Pro Asn Gly Phe Asn Gly Met Ser Val Val Phe	
	30 35 40	
	CTG GCG GGG ACC CCG GAG CAC CGC TGT CGA GTG CCG GAC GCC GCG AAC	317
5	Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn	
	45 50 55	
	CTG AGC AGC GCC TGG CGC AAC AAC AGT GTC CCG CTG CCG CTG CCG GAC	365
	Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp	
	60 65 70	
10	GGC CGC GAG GTG CCC CAC AGC TGC AGC CGC TAC CGG CTC GCC ACC ATC	413
	Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile	
	75 80 85	
	GCC AAC TTC TCG GCG CTC GGG CTG GAG CCG GGG CGC GAC GTG GAC CTG	461
	Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu	
15	90 95 100 105	
	GGG CAG CTG GAG CAG GAG AGC TGC CTG GAT GGC TGG GAG TTC AGC CAG	509
	Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln	
	110 115 120	
	GAC GTC TAC CTG TCC ACC GTC GTG ACC GAG TGG AAT CTG GTG TGT GAG	557
20	Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu	
	125 130 135	
	GAC AAC TGG AAG GTG CCC CTC ACC ACC TCC CTG TTC TTC GTA GGC GTG	605
	Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val	
	140 145 150	
25	CTC CTC GGC TCC TTC GTG TCC GGG CAG CTG TCA GAC AGG TTT GGC AGG	653
	Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg	
	155 160 165	
	AAG AAC GTT CTC TTC GCA ACC ATG GCT GTA CAG ACT GGC TTC AGC TTC	701
	Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe	
30	170 175 180 185	
	CTG CAG ATT TTC TCC ATC AGC TGG GAG ATG TTC ACT GTG TTA TTT GTC	749
	Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val	
	190 195 200	
	ATC GTG GGC ATG GGC CAG ATC TCC AAC TAT GTG GTA GCC TTC ATA CTA	797
35	Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu	
	205 210 215	

	GGA ACA GAA ATT CTT GGC AAG TCA GTT CGT ATT ATA TTC TCT ACA TTA	845
	Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu	
	220 225 230	
	GGA GTG TGC ACA TTT TTT GCA GTT GGC TAT ATG CTG CTG CCA CTG TTT	893
5	Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe	
	235 240 245	
	GCT TAC TTC ATC AGA GAC TGG CGG ATG CTG CTG CTG GCG CTG ACG GTG	941
	Ala Tyr Phe Ile Arg Asp Trp Arg Met Leu Leu Leu Ala Leu Thr Val	
	250 255 260 265	
10	CCG GGA GTG CTG TGT GTC CCG CTG TGG TGG TTC ATT CCT GAA TCT CCC	989
	Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro	
	270 275 280	
	CGA TGG CTG ATA TCC CAG AGA AGA TTT AGA GAG GCT GAA GAT ATC ATC	1037
	Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile	
15	285 290 295	
	CAA AAA GCT GCA AAA ATG AAC AAC ACA GCT GTA CCA GCA GTG ATA TTT	1085
	Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe	
	300 305 310	
	GAT TCT GTG GAG GAG CTA AAT CCC CTG AAG CAG CAG AAA GCT TTC ATT	1133
20	Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile	
	315 320 325	
	CTG GAC CTG TTC AGG ACT CGG AAT ATT GCC ATA ATG ACC ATT ATG TCT	1181
	Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser	
	330 335 340 345	
25	TTG CTG CTA TGG ATG CTG ACC TCA GTG GGT TAC TTT GCT CTG TCT CTG	1229
	Leu Leu Leu Trp Met Leu Thr Ser Val Gly Tyr Phe Ala Leu Ser Leu	
	350 355 360	
	GAT GCT CCT AAT TTA CAT GGA GAT GCC TAC CTG AAC TGT TTC CTC TCT	1277
	Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser	
30	365 370 375	
	GCC TTG ATT GAA ATT CCA GCT TAC ATT ACA GCC TGG CTG CTA TTG CGA	1325
	Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg	
	380 385 390	
	ACG CTG CCC AGG CGT TAT ATC ATA GCT GCA GTA CTG TTC TGG GGA GGA	1373
35	Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly	
	395 400 405	

GGT GTG CTT CTC TTC ATT CAA CTG GTA CCT GTG GAT TAT TAC TTC TTA 1421
 Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu
 410 415 420 425
 TCC ATT GGT CTG GTC ATG CTG GGA AAA TTT GGG ATC ACC TCT GCT TTC 1469
 5 Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
 430 435 440
 TCC ATG CTG TAT GTC TTC ACT GCT GAG CTC TAC CCA ACC CTG GTC AGG 1517
 Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
 445 450 455
 10 AAC ATG GCG GTG GGG GTC ACA TCC ACG GCC TCC AGA GTG GGC AGC ATC 1565
 Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile
 460 465 470
 ATT GCC CCC TAC TTT GTT TAC CTC GGT GCT TAC AAC AGA ATG CTG CCC 1613
 Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
 15 475 480 485
 TAC ATC GTC ATG GGT AGT CTG ACT GTC CTG ATT GGA ATC TTC ACC CTT 1661
 Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
 490 495 500 505
 TTT TTC CCT GAA AGT TTG GGA ATG ACT CTT CCA GAA ACC TTA GAG CAG 1709
 20 Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
 510 515 520
 ATG CAG AAA GTG AAA TGG TTC AGA TCT GGG AAA AAA ACA AGA GAC TCA 1757
 Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
 525 530 535
 25 ATG GAG ACA GAA GAA AAT CCC AAG GTT CTA ATA ACT GCA TTC 1799
 Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
 540 545 550
 TGAAAAATA TCTACCCCAT TTGGTGAAGT GAAAAACAGA AAAATAAGAC CCTGTGGAGA 1859
 AATTCGTTGT TCCCACTGAA ATGGACTGAC TGTAACGATT GACACCAAAA TGAACCTTGC 1919
 30 TATCAAGAAA TGCTCGTCAT ACAGTAAACT CTGGATGATT CTTCCAGATA ATGTCCTTGC 1979
 TTTACAAACC AACCATTCTT AGAGAGTCTC CTTACTCATT AATTCAATGA AATGGATTGG 2039
 TAAGATGTCT TGAAACATG TTAGTCAAGG ACTGGTAAAA TACATATAAA GATTAACACT 2099
 CATTTCGAAT CATACAAATA CTATCCAAAT AAAAAT 2135

35 SEQ ID NO: 3
 SEQUENCE LENGTH: 557

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

5 Met Arg Asp Tyr Asp Glu Val Thr Ala Phe Leu Gly Glu Trp Gly
1 5 10 15
Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu Ser Ala Ser Ile Ile Pro
20 25 30
Asn Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu
10 35 40 45
His Arg Cys Arg Val Pro Asp Ala Ala Asn Leu Ser Ser Ala Trp Arg
50 55 60
Asn His Thr Val Pro Leu Arg Leu Arg Asp Gly Arg Glu Val Pro His
65 70 75
15 Ser Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Leu
80 85 90 95
Gly Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Gln Leu Glu Gln Glu
100 105 110
Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr
20 115 120 125
Ile Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro
130 135 140
Leu Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile
145 150 155
25 Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val
160 165 170 175
Thr Met Gly Met Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys
180 185 190
Asn Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln
30 195 200 205
Ile Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly
210 215 220
Lys Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr
225 230 235
35 Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp
240 245 250 255

Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val
 260 265 270
 Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln
 275 280 285
 5 Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala
 290 295 300
 Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln
 305 310 315
 Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu
 10 320 325 330 335
 Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp
 340 345 350
 Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn
 355 360 365
 15 Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu
 370 375 380
 Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg
 385 390 395
 Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu
 20 400 405 410 415
 Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu
 420 425 430
 Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr
 435 440 445
 25 Val Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val
 450 455 460
 Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr
 465 470 475
 Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met
 30 480 485 490 495
 Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu
 500 505 510
 Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val
 515 520 525
 35 Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys
 530 535 540

Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe

545

550

555

SEQ ID NO: 4

5 SEQUENCE LENGTH: 1831

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

10 SEQUENCE

CGGACGGTCT TGGGTCGCCT GCTGCCTGGC TTGCCTGGTC GGCGGCGGGT GCGGCGCGCG 60

CACGCGCAAA GCGGCGCGCG TTCCCAGACC CCAGGCGCGG CTCTGTGGGC CTCTGAGGGC 120

GGC ATG CGG GAC TAC GAC GAG GTG ACC GCC TTC CTG GGC GAG TGG GGG 168

Met Arg Asp Tyr Asp Glu Val Thr Ala Phe Leu Gly Glu Trp Gly

15 1 5 10 15

CCC TTC CAG CGC CTC ATC TTC TTC CTG CTC AGC GCC AGC ATC ATC CCC 216

Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu Ser Ala Ser Ile Ile Pro

20 25 30

AAT GGC TTC ACC GGC CTG TCC TCC GTG TTC CTG ATA GCG ACC CCG GAG 264

20 Asn Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu

35 40 45

CAC CGC TGC CGG GTG CCG GAC GCC GCG AAC CTG AGC AGC GCC TGG CGC 312

His Arg Cys Arg Val Pro Asp Ala Ala Asn Leu Ser Ser Ala Trp Arg

50 55 60

25 AAC CAC ACT GTC CCA CTG CGG CTG CGG GAC GGC CGC GAG GTG CCC CAC 360

Asn His Thr Val Pro Leu Arg Leu Arg Asp Gly Arg Glu Val Pro His

65 70 75

AGC TGC CGC CGC TAC CGG CTC GCC ACC ATC GCC AAC TTC TCG GCG CTC 408

Ser Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Leu

30 80 85 90 95

GGG CTG GAG CCG GGG CGC GAC GTG GAC CTG GGG CAG CTG GAG CAG GAG 456

Gly Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Gln Leu Glu Gln Glu

100 105 110

AGC TGT CTG GAT GGC TGG GAG TTC AGT CAG GAC GTC TAC CTG TCC ACC 504

35 Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr

115 120 125

	ATT GTG ACC GAG TGG AAC CTG GTG TGT GAG GAC GAC TGG AAG GCC CCA	552
	Ile Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro	
	130 135 140	
	CTC ACA ATC TCC TTG TTC TTC GTG GGT GTG CTG TTG GGC TCC TTC ATT	600
5	Leu Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile	
	145 150 155	
	TCA GGG CAG CTG TCA GAC AGG TTT GGC CGG AAG AAT GTG CTG TTC GTG	648
	Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val	
	160 165 170 175	
10	ACC ATG GGC ATG CAG ACA GGC TTC AGC TTC CTG CAG ATC TTC TCG AAG	696
	Thr Met Gly Met Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys	
	180 185 190	
	AAT TTT GAG ATG TTT GTC GTG CTG TTT GTC CTT GTA GGC ATG GGC CAG	744
	Asn Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln	
15	195 200 205	
	ATC TCC AAC TAT GTG GCA GCA TTT GTC CTG GGG ACA GAA ATT CTT GGC	792
	Ile Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly	
	210 215 220	
	AAG TCA GTT CGT ATA ATA TTC TCT ACG TTA GGA GTG TGC ATA TTT TAT	840
20	Lys Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr	
	225 230 235	
	GCA TTT GGC TAC ATG GTG CTG CCA CTG TTT GCT TAC TTC ATC CGA GAC	888
	Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp	
	240 245 250 255	
25	TGG CGG ATG CTG CTG GTG GCG CTG ACG ATG CCG GGG GTG CTG TGC GTG	936
	Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val	
	260 265 270	
	GCA CTC TGG TGG TTC ATC CCT GAG TCC CCC CGA TGG CTC ATC TCT CAG	984
	Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln	
30	275 280 285	
	GGA CGA TTT GAA GAG GCA GAG GTG ATC ATC CGC AAG GCT GCC AAA GCC	1032
	Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala	
	290 295 300	
	AAT GGG ATT GTT GTG CCT TCC ACT ATC TTT GAC CCG AGT GAG TTA CAA	1080
35	Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln	
	305 310 315	

	GAC CTA AGT TCC AAG AAG CAG CAG TCC CAC AAC ATT CTG GAT CTG CTT	1128
	Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu	
	320 325 330 335	
	CGA ACC TGG AAT ATC CGG ATG GTC ACC ATC ATG TCC ATA ATG CTG TGG	1176
5	Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp	
	340 345 350	
	ATG ACC ATA TCA GTG GGC TAT TTT GGG CTT TCG CTT GAT ACT CCT AAC	1224
	Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn	
	355 360 365	
10	TTG CAT GGG GAC ATC TTT GTG AAC TGC TTC CTT TCA GCG ATG GTT GAA	1272
	Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu	
	370 375 380	
	GTC CCA GCA TAT GTG TTG GCC TGG CTG CTG CTG CAA TAT TTG CCC CGG	1320
	Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg	
15	385 390 395	
	CGC TAT TCC ATG GCC ACT GCC CTC TTC CTG GGT GGC AGT GTC CTT CTC	1368
	Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu	
	400 405 410 415	
	TTC ATG CAG CTG GTA CCC CCA GAC TTG TAT TAT TTG GCT ACA GTC CTG	1416
20	Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu	
	420 425 430	
	GTG ATG GTG GGC AAG TTT GGA GTC ACG GCT GCC TTT TCC ATG GTC TAC	1464
	Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr	
	435 440 445	
25	GTG TAC ACA GCC GAG CTG TAT CCC ACA GTG GTG AGA AAC ATG GGT GTG	1512
	Val Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val	
	450 455 460	
	GGA GTC AGC TCC ACA GCA TCC CGC CTG GGC AGC ATC CTG TCT CCC TAC	1560
	Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr	
30	465 470 475	
	TTC GTT TAC CTT GGT GCC TAC GAC CGC TTC CTG CCC TAC ATT CTC ATG	1608
	Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met	
	480 485 490 495	
	GGA AGT CTG ACC ATC CTG ACA GCC ATC CTC ACC TTG TTT CTC CCA GAG	1656
35	Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu	
	500 505 510	

AGC TTC GGT ACC CCA CTC CCA GAC ACC ATT GAC CAG ATG CTA AGA GTC 1704
 Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val
 515 520 525
 AAA GGA ATG AAA CAC AGA AAA ACT CCA AGT CAC ACA AGG ATG TTA AAA 1752
 5 Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys
 530 535 540
 GAT GGT CAA GAA AGG CCC ACA ATC CTT AAA AGC ACA GCC TTC 1794
 Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
 545 550 555
 10 TAACATCGCT TCCAGTAAGG GAGAACTGA AGAGGAA 1831

SEQ ID NO: 5

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

15 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE

CTAATACGAC TCACTATAGG GC 22

20

SEQ ID NO: 6

SEQUENCE LENGTH: 21

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE

TGTAGCGTGA AGACGACAGA A 21

30 SEQ ID NO: 7

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

35 MOLECULE TYPE: other nucleic acid, synthetic DNA

SEQUENCE

TCGAGCGGCC GCCCGGCAG GT

22

SEQ ID NO: 8

SEQUENCE LENGTH: 22

5 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

10 AGGGCGTGGT GCGGAGGGCG GT

22

SEQ ID NO: 9

SEQUENCE LENGTH: 20

SEQUENCE TYPE: nucleic acid

15 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

CTTTGAGCA AGTTCAGCCT

20

20

SEQ ID NO: 10

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

AGAGGTGGCT TATGAGTATT TCTT

24

30 SEQ ID NO: 11

SEQUENCE LENGTH: 22

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

35 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

CCAGGGTTTT CCCAGTCACG AC

22

SEQ ID NO: 12

SEQUENCE LENGTH: 22

5 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

10 TCACACAGGA AACAGCTATG AC

22

SEQ ID NO: 13

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

15 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

GTGCTGTTGG GCTCCTTCAT TTCA

24

20

SEQ ID NO: 14

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

AGCTGCATGA AGAGAAGGAC ACTG

24

30 SEQ ID NO: 15

SEQUENCE LENGTH: 24

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

35 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

AGCATCCTGT CTCCTACTT CGTT

24

SEQ ID NO: 16

SEQUENCE LENGTH: 33

5 SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

10 GATGGATCCC GGACGGTCTT GGGTCGCCTG CTG

33

SEQ ID NO: 17

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

15 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

GATGGATCCA AATGCTGCCA CATAGTTGGA GAT

33

20

SEQ ID NO: 18

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

25 TOPOLOGY: linear

MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

GATGGATCCA TGGGCATGCA GACAGGCTTC AGC

33

30 SEQ ID NO: 19

SEQUENCE LENGTH: 33

SEQUENCE TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

35 MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

GATGGATCCT TCCTCTTCAG TTTCTCCCTT ACT

33

[Brief Description of the Drawings]

[Figure 1] It represents hydrophobicity plots of OCTN1 and OCTN2 according to Kyte & Doolittle's calculating formula with a window of nine amino acid residues. Numerals on the plots indicate putative transmembrane regions.

[Figure 2] It represents electrophoretic patterns showing the results of Northern blot analysis of OCTN1.

[Figure 3] It compares the amino acid sequence of OCTN1 with that of OCTN2. Amino acid residues conserved in both transporters are shaded. Sequences coinciding with the consensus sequences of sugar transporter and the ATP/GTP binding site are indicated by "+" and "*", respectively.

[Figure 4] It represents electrophoretic patterns showing the results of Northern blot analysis of OCTN2.

[Figure 5] It is a graph showing the TEA-absorbing activity of OCTN1. Clear circles represent untreated cells, and solid circles represent OCTN1-transfected cells.

[Figure 6] It is a graph showing effects of the cold TEA added in the experimental system in Fig. 5. In this graph, solid circles represent OCTN1-transfected cells, and clear circles represent cells containing the vector with no insert. Clear triangles indicate the net uptake induced by OCTN1 obtained by subtracting the clear circle values from the corresponding solid circle values.

[Figure 7] It is a graph showing TEA concentration-dependency of the TEA-absorbing activity of OCTN1.

[Figure 8] It is a bar graph showing the activity of the OCTN1-transfected cells to absorb substances other than TEA.

[Figure 9] It is a bar graph showing the results of transport experiments using *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the OCTN1-injected cRNA oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of TEA, carnitine, mepyramine, quinidine, and actinomycin D were observed in OCTN1 cRNA-injected oocytes, whereas water-injected oocytes (containing no cRNA)

exhibited almost no uptake activity.

[Figure 10] It is a bar graph showing the results of transport experiments for carcinostatics in *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the OCTN1 cRNA-injected oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of actinomycin D, etoposide, vinblastine, and daunomycin were observed in the OCTN1 cRNA-injected oocytes.

[Figure 11] It is a bar graph showing the results of transport experiments with OCTN1 and OCTN2 in HEK293 cells. OCTN1 has the efficient transport activity for TEA and OCTN2 for carnitine.

[Figure 12] It is a graph showing the results of Na^+ -dependency of the carnitine transport activity of OCTN2. OCTN2 exhibits a time-dependent carnitine transport activity (clear circle) in the presence of Na^+ , while no such activity in the absence of Na^+ (solid circle), indicating that the carnitine transport activity of OCTN2 depends on the presence of Na^+ .

Drawings

Figure 1

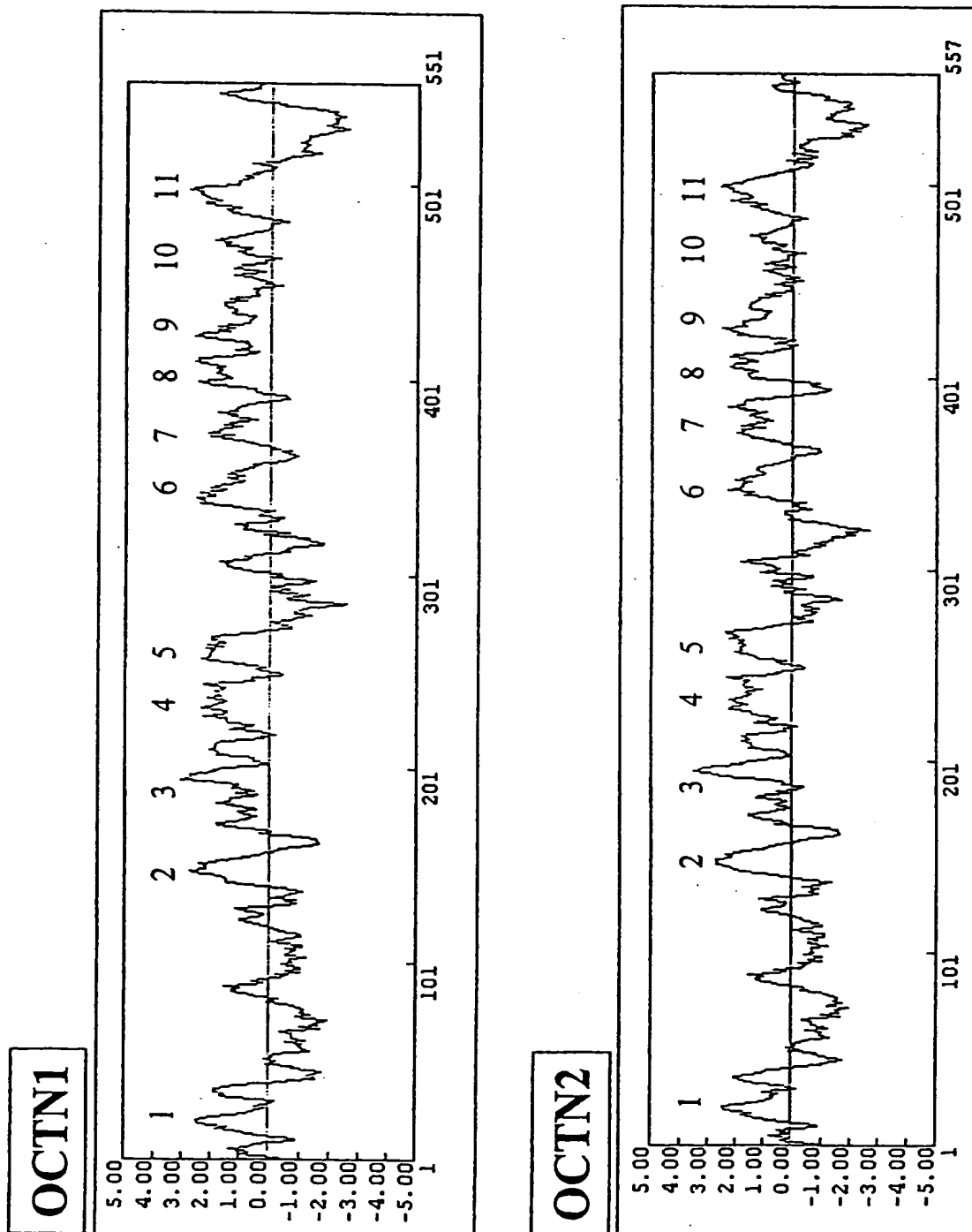


Figure 2

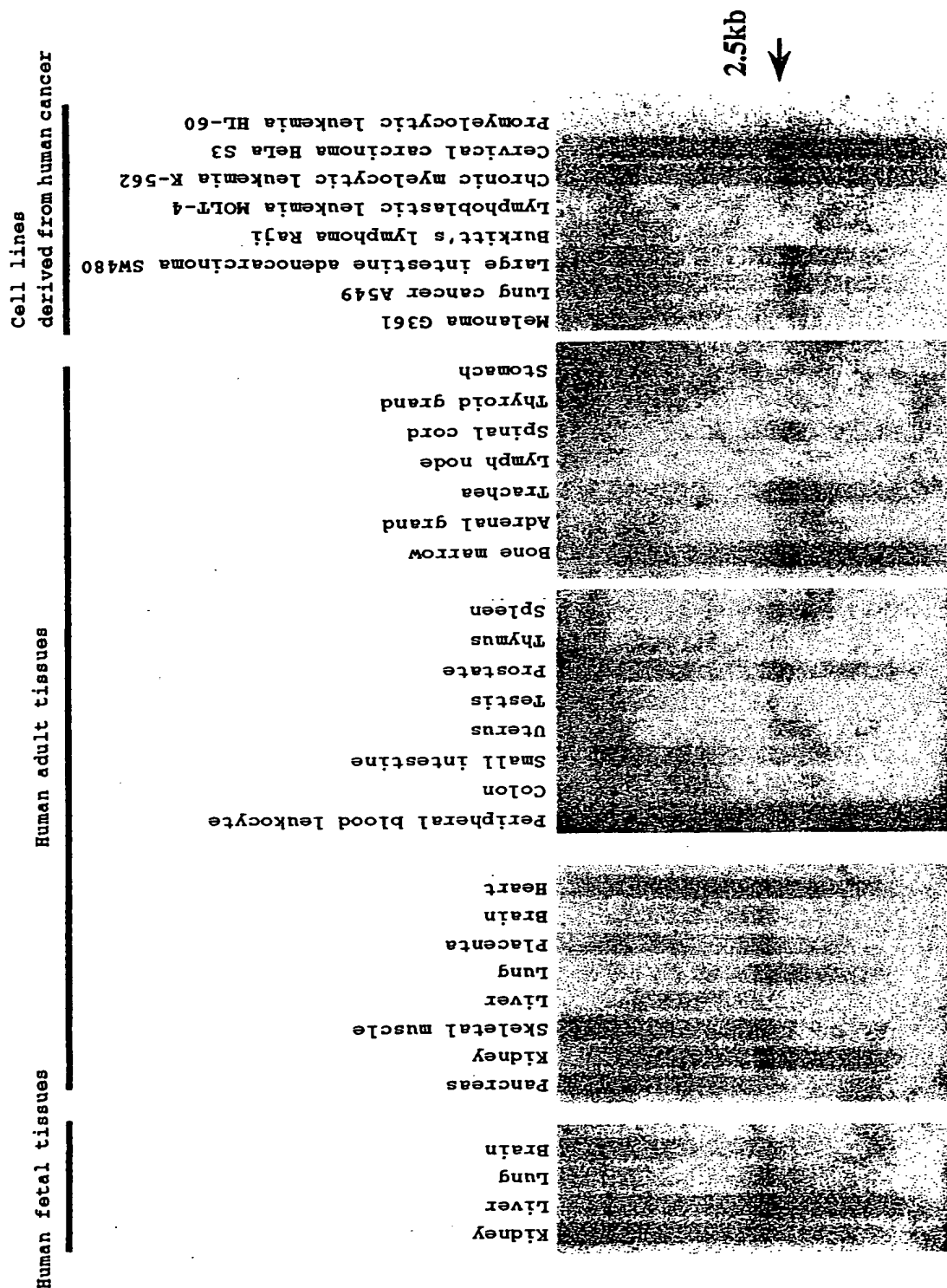


Figure 3

```

OCTN1 1 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 1 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

OCTN1 101 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 101 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

OCTN1 201 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 201 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

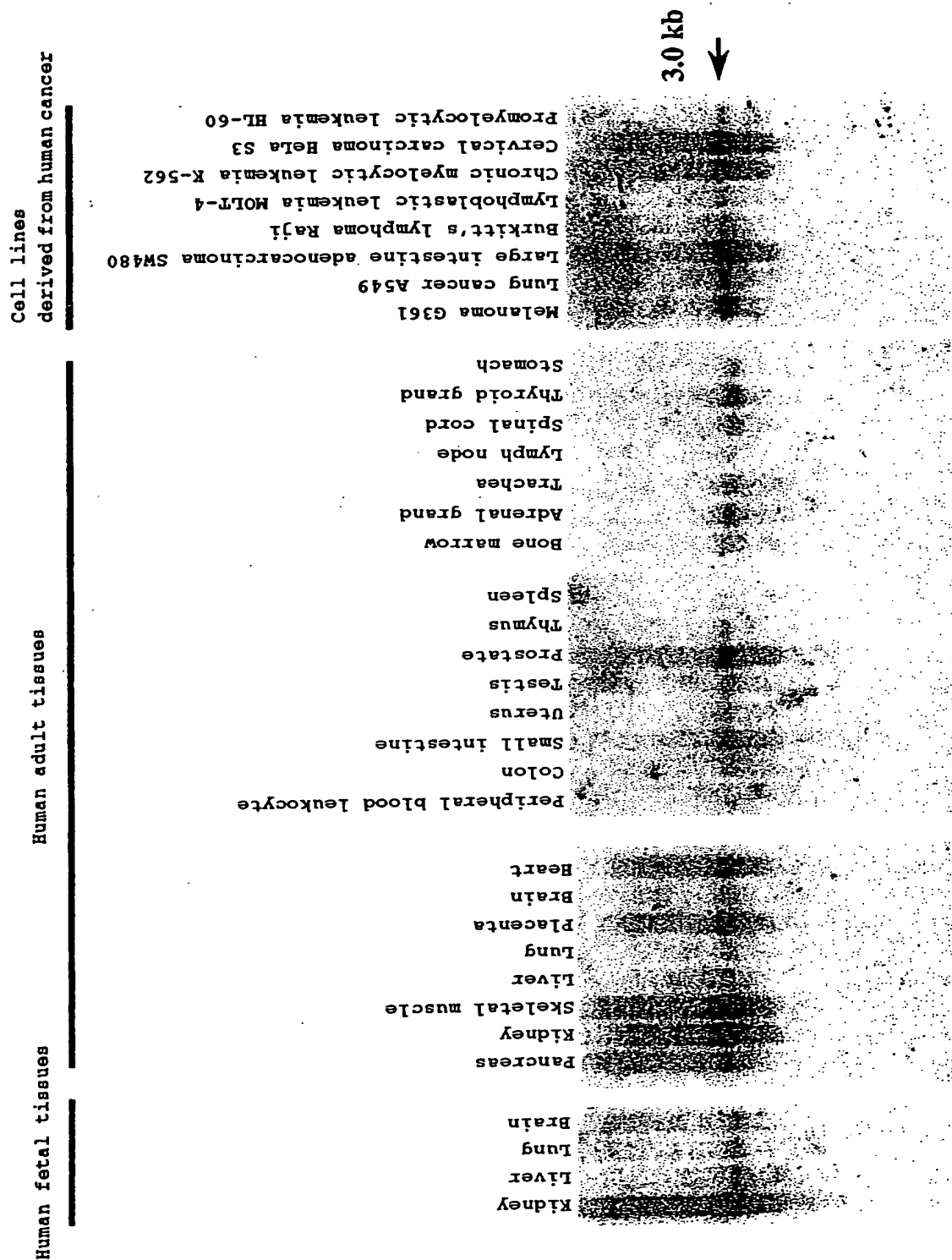
OCTN1 301 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 301 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

OCTN1 399 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 401 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

OCTN1 499 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG
OCTN2 501 1RDNDENIAT 1GCHGQFQRI 1PEPLSSKSI 1AGCNQNSVI 1EAGCHPERG 1RUPDANLSS 1APRNSVPER 1FRKNGKAPIS 1SRVRCATKA 1NFSALCLEPG

```

Figure 4



Reference No. = C2-906DP1

(5/12)

Figure 5

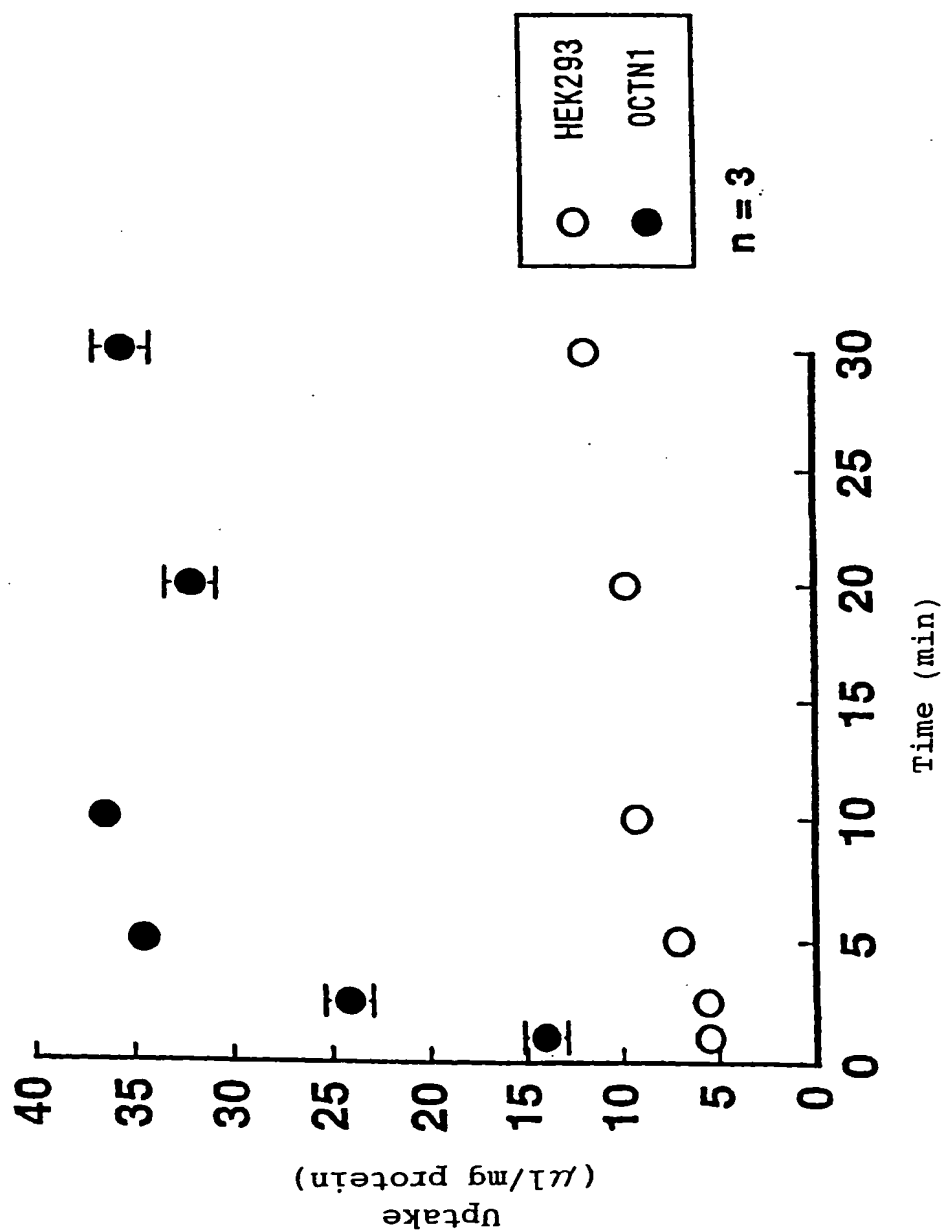
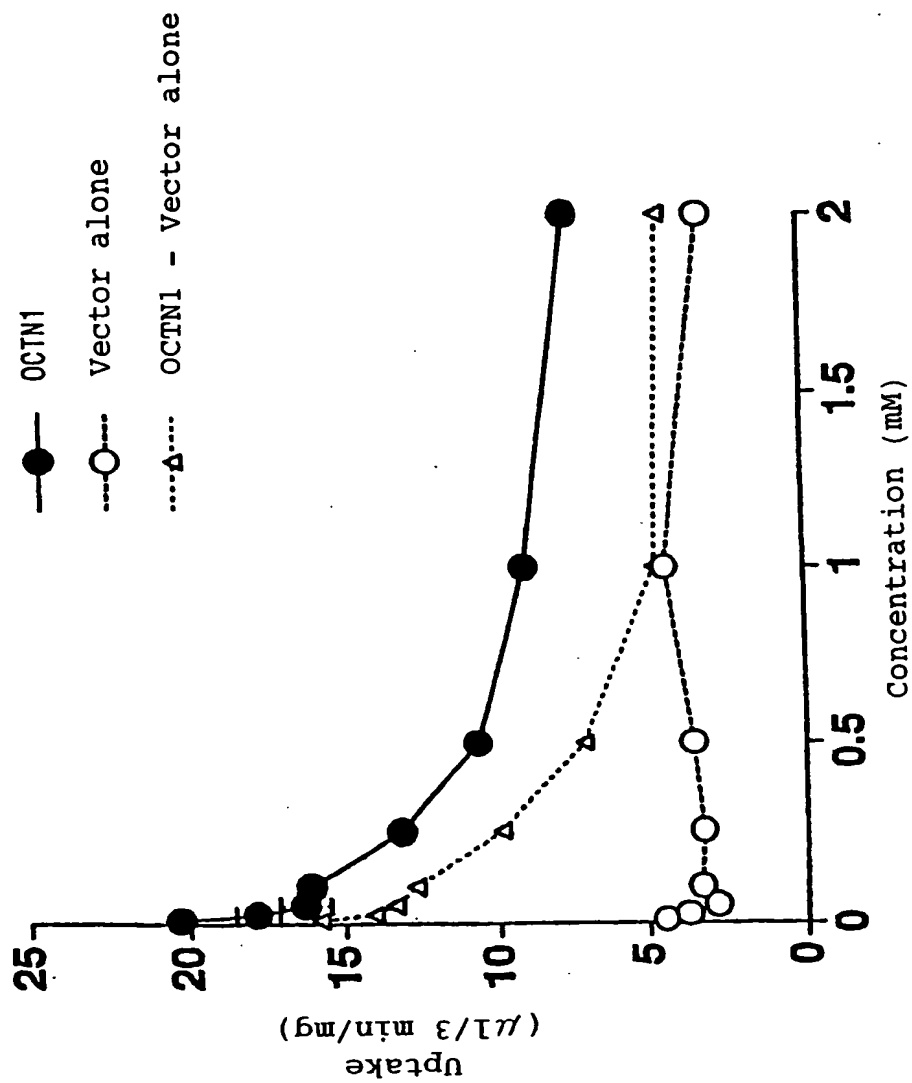


Figure 6



Reference No. = C2-906DP1

(7/12)

Figure 7

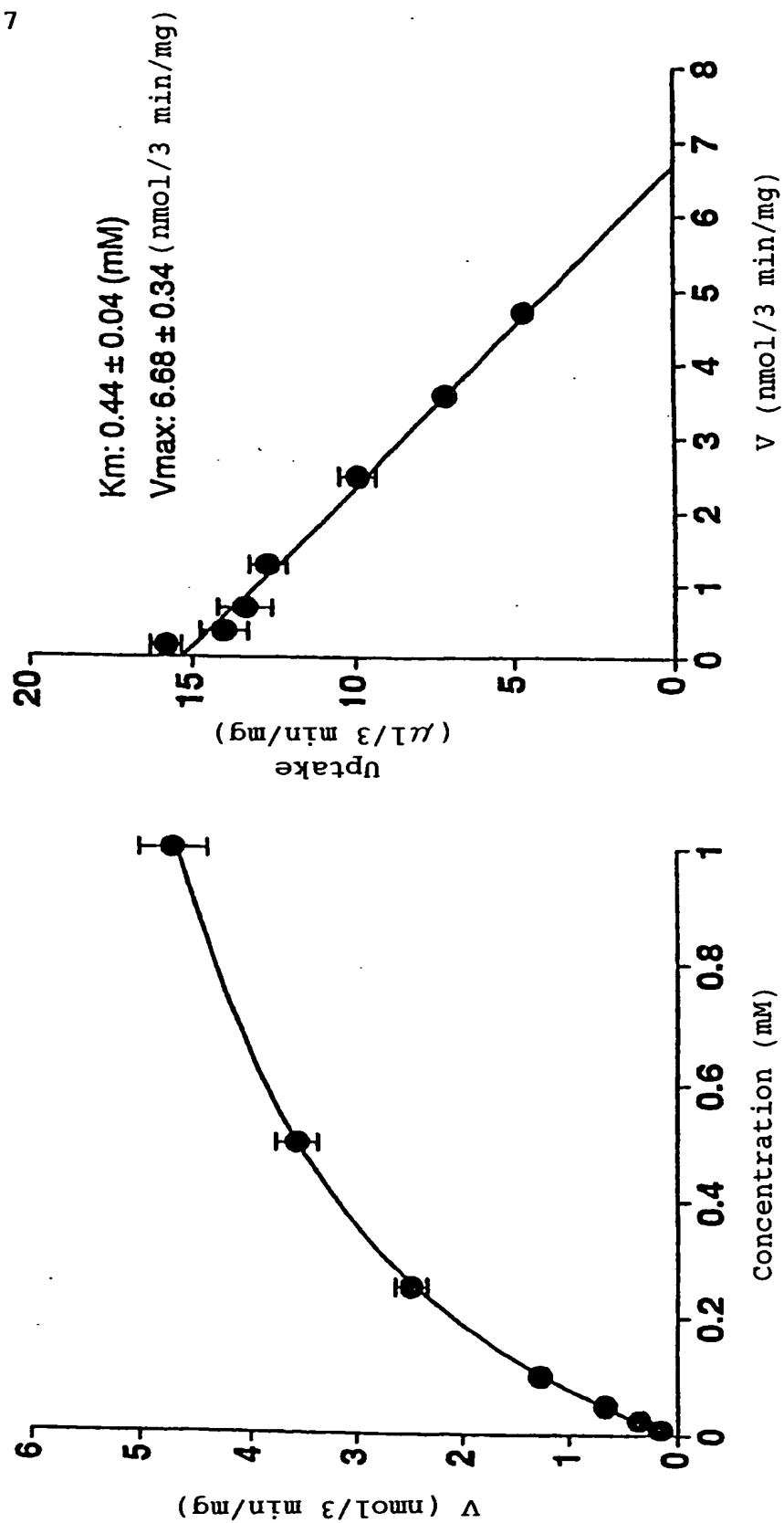


Figure 8

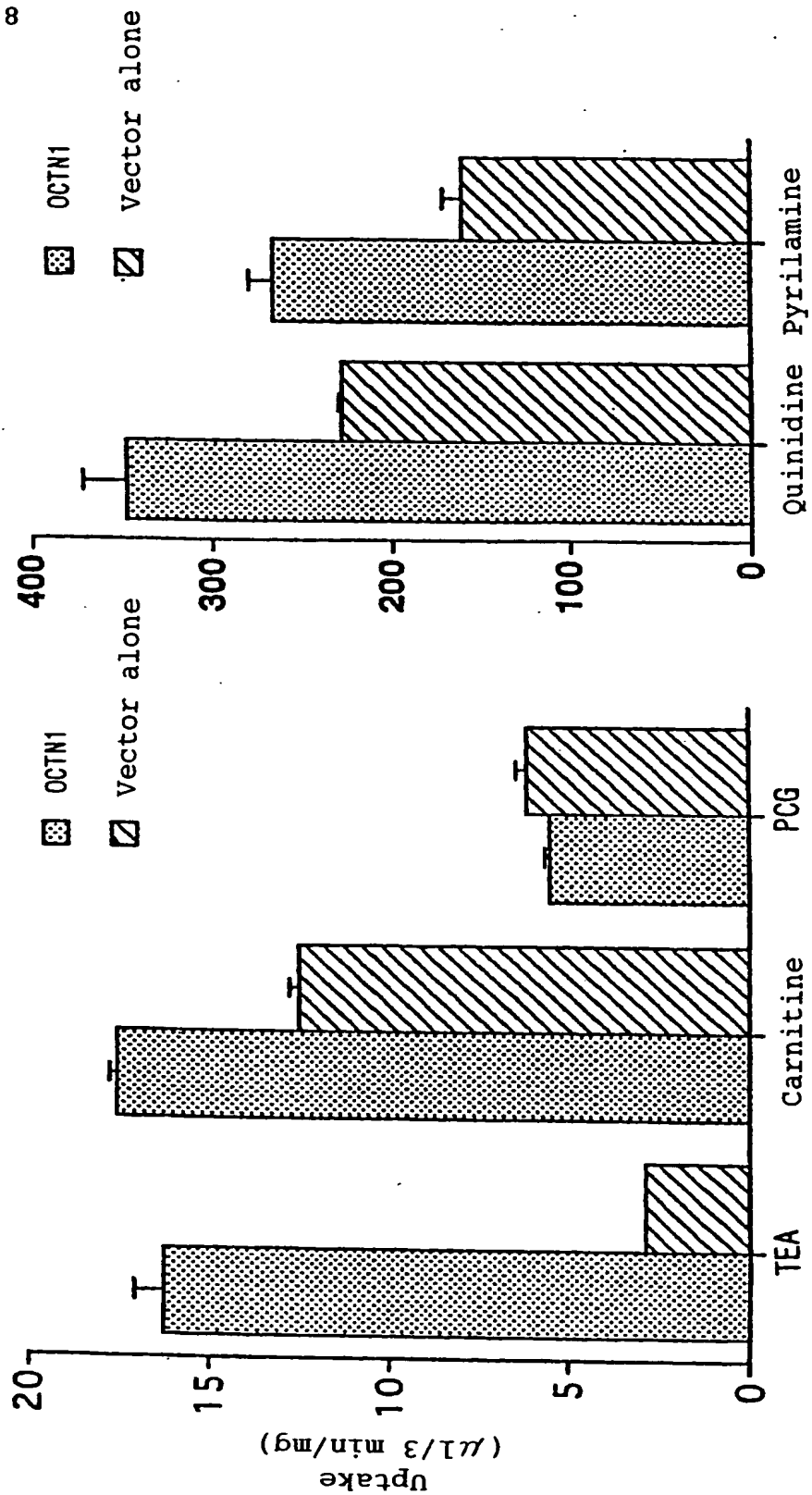


Figure 9

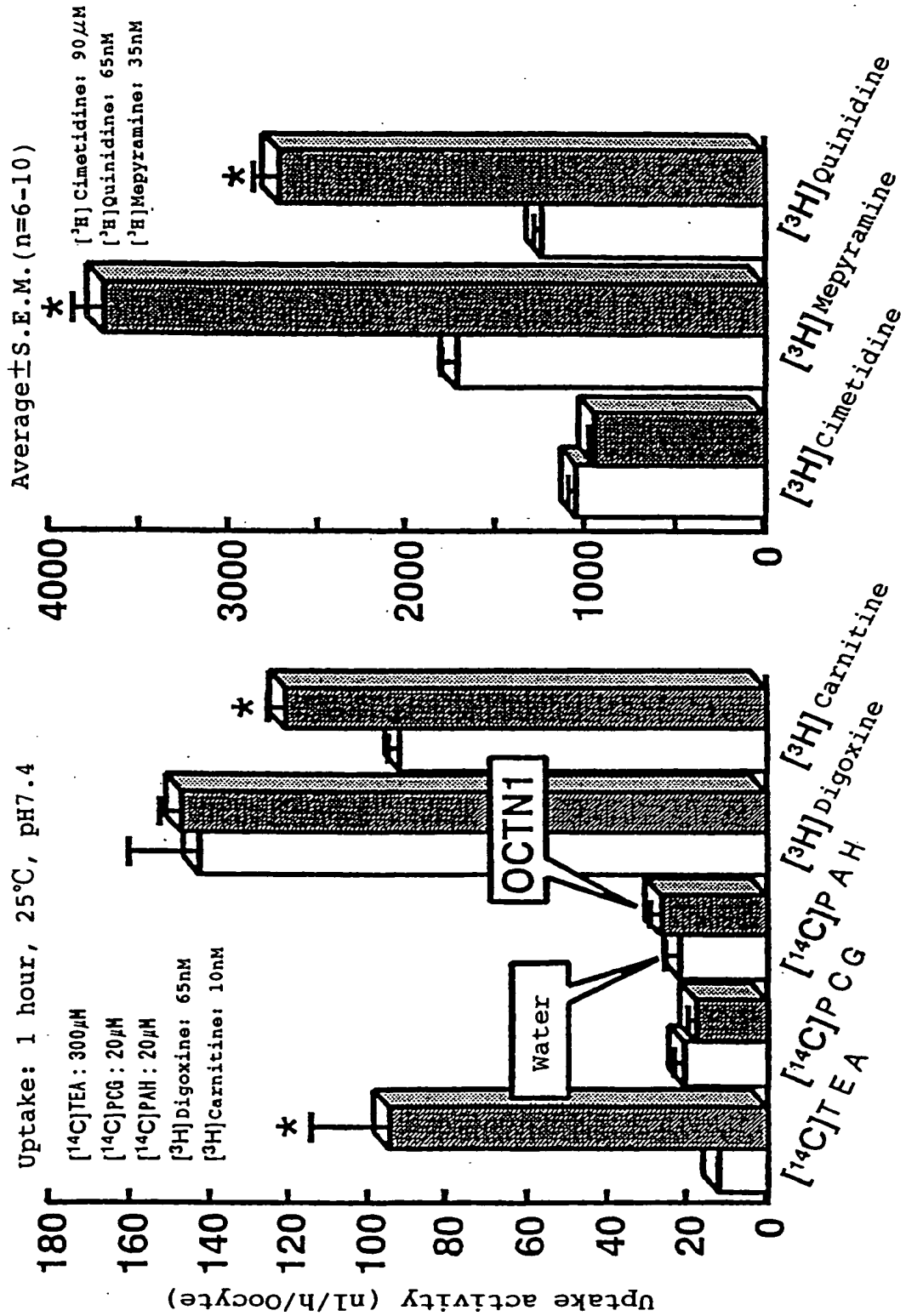


Figure 10

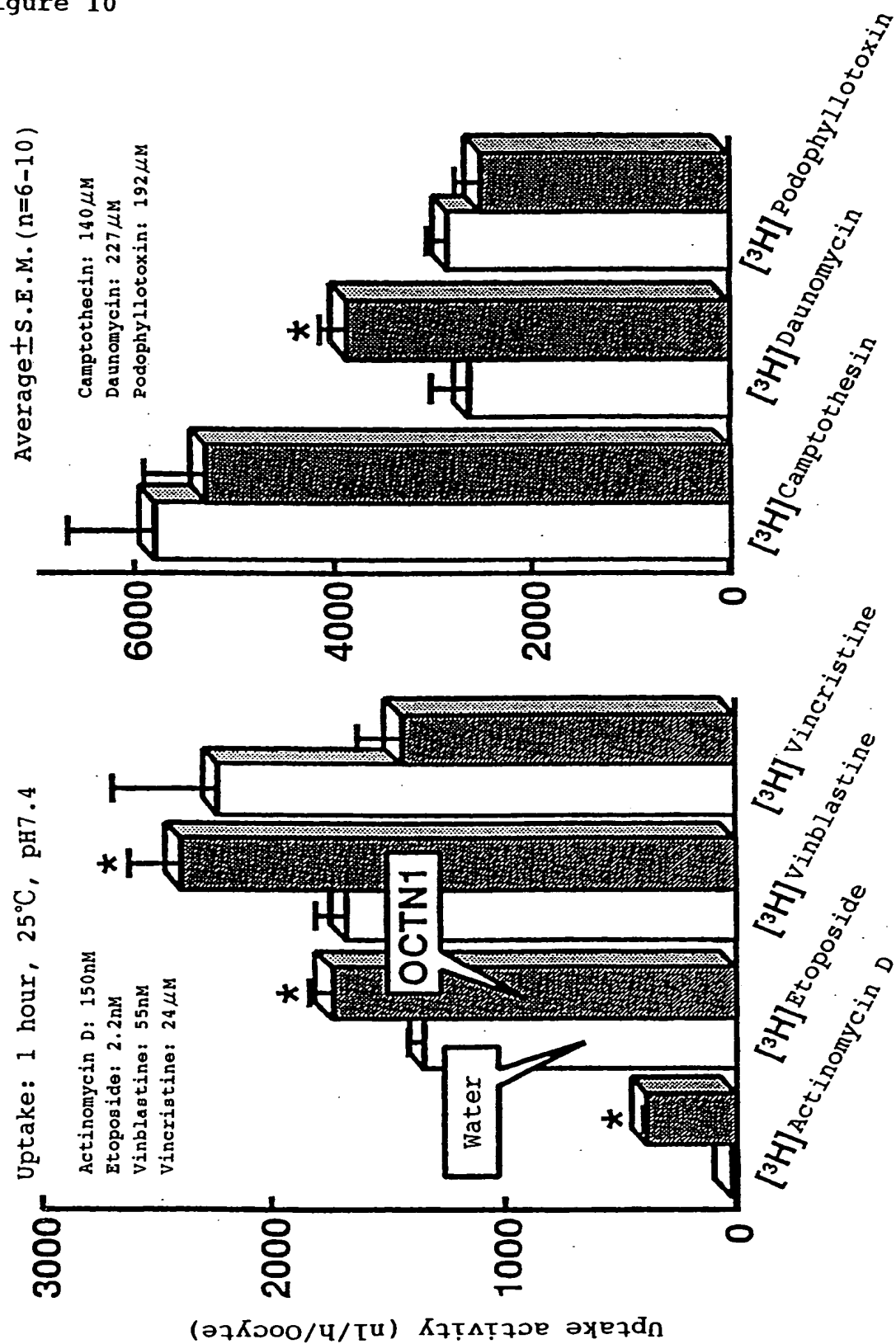
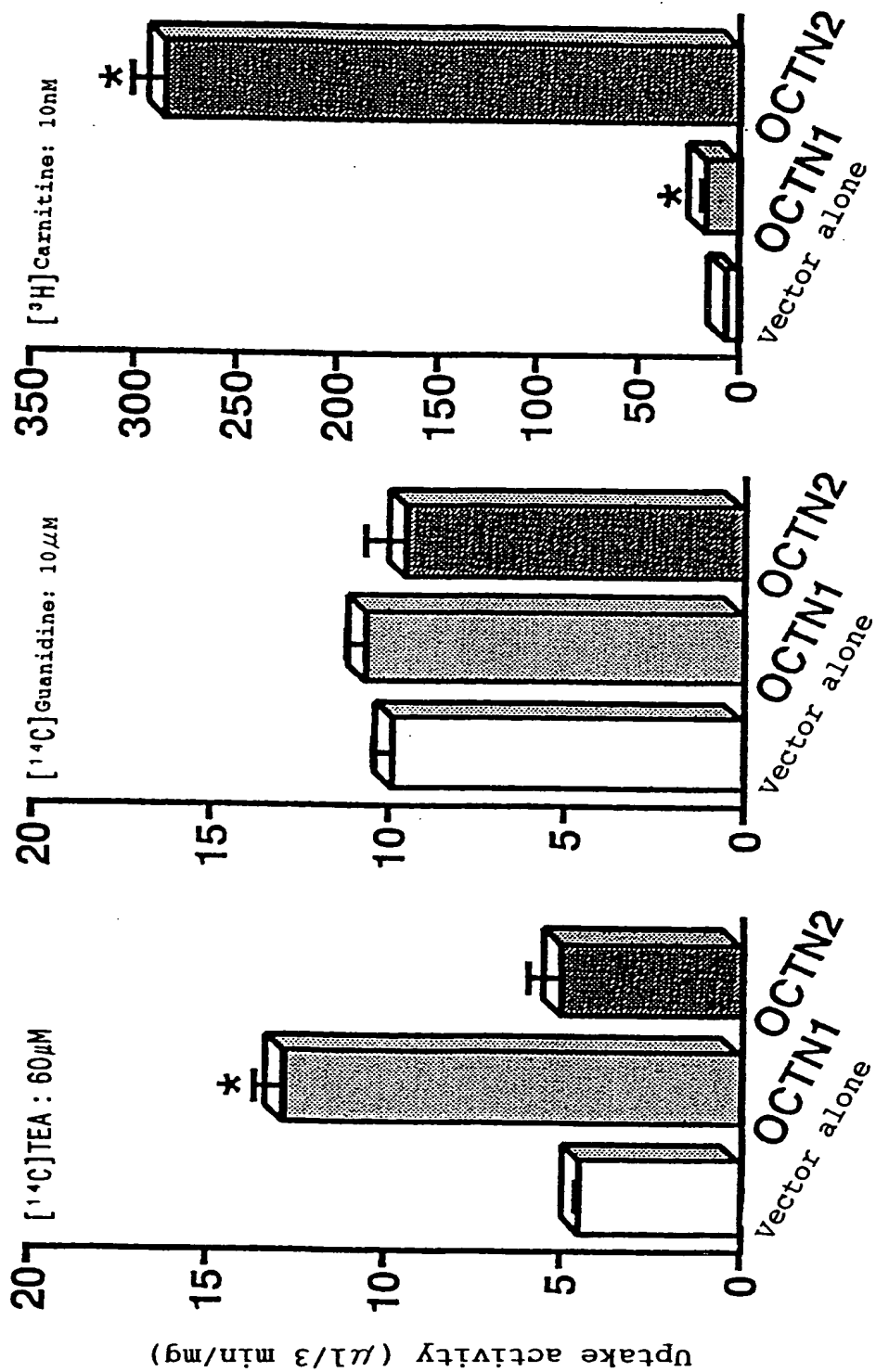


Figure 11



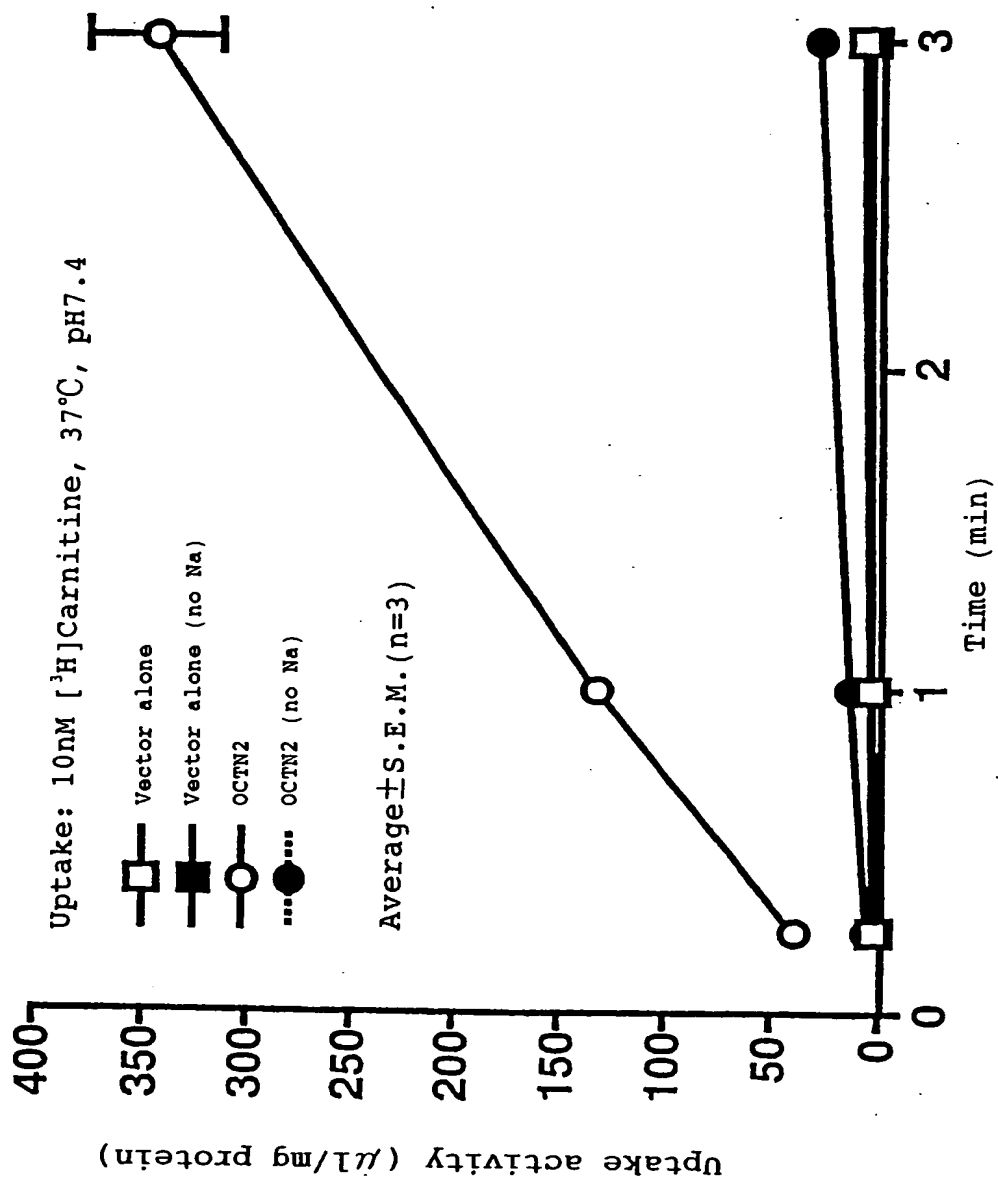
Uptake: 3 min, 37°C, pH7.4

Average ± S.E.M. (n=3)

Reference No. = C2-906DP1

(12/12)

Figure 12



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.